Abnormal Ductus Venosus Flow and Tricuspid Regurgitation at 11–14 Weeks' Gestation have High Positive Predictive Values for Increased Risk in First-trimester Combined Screening Test: Results of a Pilot Study

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SUMMARY

Objective: To investigate the relationship between two novel first-trimester ultrasound markers (abnormal fetal ductus venosus [DV] flow and presence of tricuspid regurgitation [TR]) and the results of the first-trimester combined screening test in pregnancies with a normal karyotype. The screening test involves nuchal translucency measurement by ultrasound, and measurement of serum free β-chorionic gonadotropin and pregnancy-associated plasma protein A.

Materials and Methods: The study included 58 pregnancies with amniocentesis-proven normal karyotypes and ultrasound-proven normal fetal anatomy. DV flow and TR were initially evaluated by ultrasound at 11–14 weeks' gestation. Sensitivity, specificity, and positive and negative predictive values of abnormal DV flow and TR for determining increased test risk (> 1 in 300) were calculated.

Results: Abnormal DV flow and TR were detected in seven (12%) and six (10%) women, respectively. The sensitivities of abnormal DV flow, TR, and dual abnormalities (abnormal DV flow plus TR) for predicting increased risk in the combined screening test were low (33.3%, 27.7%, and 26.3%, respectively). However, their corresponding specificities (97.5%, 97.5%, and 100%) and positive predictive values (85.7%, 83.3%, and 100%) were reasonably high, with particularly low false-positive rates (2.5%, 2.5%, and 0%). When abnormal DV flow and TR were both positive, the combined test risk was consistently above 1 in 300.

Conclusion: Determination of DV flow and TR as initial markers in unselected pregnancies merits further investigation, as the combination of these parameters might reliably predict an increased risk in combined screening test result, with low false positivity. [*Taiwan J Obstet Gynecol* 2010;49(2):145–150]

Key Words: combined screening test, ductus venosus, tricuspid regurgitation

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Introduction

One of the fundamental roles of prenatal diagnosis is the timely antenatal detection of common chromosomal pathologies, such as trisomy 21 [1]. However, the exact diagnosis of fetal chromosomal abnormalities requires cytogenetic studies. Although noninvasive diagnosis using fetal cells from maternal plasma and nucleic acid assessment has been under investigation for almost

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three decades, these technologies are still not in routine clinical use [2]. Chorionic villus sampling and genetic amniocentesis, therefore, continue to provide the mainstay of prenatal diagnosis for chromosomal defects, until noninvasive techniques become adopted for routine clinical use.

The major aim of antenatal screening tests is to decrease the rate of invasive diagnostic procedures. Current screening tests generally include biochemical assays and assessment of ultrasound markers. Biochemical assessments include measurements of pregnancy-associated plasma protein A (PAPP-A) and free β-chorionic gonadotropin (β-hCG) in the first trimester, and α-fetoprotein, β-hCG and estriol in the second trimester.

Conventional combined first-trimester screening (nuchal translucency [NT] plus PAPP-A plus free β-hCG) has been considered to be an accurate test for the detection of trisomy 21, with 90% sensitivity and a 5% false-positive rate. Additional ultrasound markers have recently been added to the tests, with the aim of further reducing the invasive procedure rate. These include NT, presence/absence of nasal bone, frontomaxillary facial angle, normal/abnormal *a* wave in ductus venosus (DV) Doppler flow analysis, and presence/absence of tricuspid regurgitation (TR) [3–8].

The addition of DV Doppler flow analysis and TR to the test results in similar sensitivity (about 90%), but appears to improve the false-positive rate (2–9%) for trisomy 21 detection [8,9]. Although DV and TR have been evaluated as adjuncts to first-trimester screening tests, their use as an initial step in the screening algorithm is not clear. The present pilot study aimed to analyze the relationship between these two novel ultrasound markers (DV and TR) and the results of the first-trimester combined test (with NT, PAPP-A, and free β-hCG) in singleton pregnancies with normal karyotypes.

Materials and Methods

The initial study included low-risk singleton pregnancies at 11^{+0} to 13^{+6} weeks of gestation during an 18-month period. Women were initially scanned by ultrasound to determine crown-rump length and basic anatomy. NT measurements, DV Doppler flow analysis and pulsed Doppler analysis for TR were then obtained. The individual risks were calculated based on NT values combined with maternal serum PAPP-A and free β-hCG measurements taken on the day of the ultrasound scan. DV and TR results were not used to calculate the combined risk. The study was subject to local ethics review committee approval.

The aim of the present pilot study was to evaluate only healthy pregnancies with no karyotype or structural abnormalities. We, therefore, excluded any pregnancies if: (1) the fetal NT measurement was > 2.5 mm; (2) any structural abnormalities were detected or suspected on ultrasound at 11–14 or 21–23 weeks' gestation (including second-trimester markers for aneuploidy, such as increased nuchal fold (> 6 mm), short long bones (< 5^{th} percentile), and single umbilical artery); (3) normal fetal karyotype results could not be confirmed or were unavailable; or (4) routine follow-up until term was missing. Karyotype abnormalities were excluded by amniocentesis (at 16–19 weeks of gestation). Chorionic villus sampling was not routinely performed during the study period.

Initially, 1,050 women scanned in the 11^{+0} to 13^{+6} weeks of pregnancy were enrolled during the 18-month period. After exclusions, 66 women with fetal karyotype reports were available. Eight women were further excluded; one had confirmed aneuploidy (trisomy 21), and seven others were lost to follow-up after 20 weeks' gestation. The remaining 58 women comprised our study group.

All ultrasound scans were performed by one of the investigators (O.O.) using the same machine (GE Voluson 730 Expert; GE Medical Systems Kretztechnik GmbH & Co, Zipf, Austria). NT measurements were obtained as defined previously [10]. The midsagittal plane view and fetal tissue–amniochorionic membrane discrimination were meticulously visualized. DV Doppler waveform analyses were performed as previously described [11]. Briefly, the waveform was obtained at the right ventral midsagittal plane during fetal quiescence using pulsed Doppler flow. If necessary, color Doppler mapping of the DV was obtained. Discrimination of the inferior vena cava, left hepatic vein and intrahepatic portion of the umbilical vein was specifically considered. DV flow was recorded as "normal" or "abnormal". Absence of or reversed flow during atrial contraction (*a* wave) was considered as abnormal.

TR was determined as described previously [10,12]. Briefly, a four-chamber view of the heart, aortic outflow and axis were first visualized. A pulsed Doppler gate was then located towards the apical mid-portion of the tricuspid valve in the four-chamber view, and the Doppler waveform was obtained. Three consecutive measurements were made. The diagnosis of TR required two distinct criteria to be met: (1) regurgitation during at least half of the systole, and (2) regurgitating flow rate ≥ 60 cm/s. Other details of the technique have been described elsewhere [10].

PAPP-A and free β-hCG measurements were performed using an Immulite 2000 Immunoassay System

(Siemens Healthcare Diagnostics, Deerfield, IL, USA), using L2KPC2 and L2KFB2 assay kits. PRISCA Prenatal Risk Calculation Software version 4.0 (Siemens Healthcare Diagnostics) was used to evaluate the risk.

The decision to perform an invasive procedure (i.e. amniocentesis) depended on the woman's decision following a detailed consultation with both partners based on the first-trimester screening results. This included information on trisomy 21, evaluation of the test results, and information on the risks involved in the invasive procedure. The decision to perform an invasive procedure did not depend solely on any specific cut-off value. The women and their partners signed a written consent form. Amniocentesis procedures were performed abdominally under ultrasound guidance using a 22-gauge needle. About 20 mL of amniotic fluid was aspirated and sent for karyotype analysis by routine cell culture. Women in the study group also underwent ultrasound scanning at 21–23 weeks' gestation. This included detailed fetal anatomy and fetal cardiac scanning. Four-chamber view, three-vessel view, outflow tracts, arterial duct, and aortic arch were routinely visualized.

Correlation between DV-tricuspid valve Doppler flow results and the reported risk in the combined test (NT plus PAPP-A plus free β-hCG) at 11–14 weeks' gestation was examined in 58 singleton pregnancies with amniocentesis-proven normal karyotypes and ultrasound-proven normal fetal anatomy. The sensitivity, specificity, positive predictive value (PPV), negative predictive value, false-negative rate, false-positive rate, positive likelihood ratio, negative likelihood ratio, and accuracy rate of abnormal DV flow and TR for determining a screen-positive test result were calculated (cut-off value, > 1 in 300). Statistical analyses were performed using the "descriptive tests module" in SPSS version 15.0 software (SPSS, Chicago, IL, USA).

Results

The mean (± standard deviation) age, gravidity, and parity were 30.2 ± 6.2 years, 1.9 ± 0.9, and 0.7 ± 0.7, respectively ($n = 58$). Indications for genetic amniocentesis are shown in Table 1. Advanced maternal age and maternal anxiety were the most frequent indications (53%).

Abnormal DV flow and TR were present in 12% (7 of 58) and 10% (6 of 58) of pregnancies, respectively. Five fetuses had abnormal DV flow accompanied by TR. Two pregnancies had abnormal DV flow and normal tricuspid flow. One case had TR but normal DV flow.

Table 1. Indications for invasive testing for karyotype analysis

Indication	*n* (%)
Advanced maternal age and/or maternal anxiety	31 (53)
Increased risk in combined screening test	18 (31)
Previous infant with malformation	3 (5)
Previous infant with trisomy 21	3 (5)
Balanced translocation in parent	1 (2)
Determination of acetylcholinesterase	1 (2)
Previous infant with DMD	1 (2)
Total	58 (100)

DMD = Duchenne muscular dystrophy.

Table 2. Combined test risk in patients with ductus venosus (DV) waveform, tricuspid regurgitation (TR), and abnormal DV flow plus TR

	Combined test risk	
	> 1 in 300	< 1 in 300
DV flow		
Abnormal	6	1
Normal	12	39
TR		
Present	5	1
Absent	13	39
Abnormal DV flow + TR		
Both abnormal	5	–
Other	14	39

The distributions of pregnancies with abnormal DV flow, presence of TR, and presence of abnormal DV flow accompanied by TR are given in Table 2. Table 3 summarizes the efficacy of the studied ultrasound parameters for the prediction of increased risk (> 1 in 300) in the first-trimester screening test. Generally, abnormal DV flow and TR had low sensitivities (33.3%, and 27.7%, respectively) but exceedingly high specificities (both 97.5%) with low false-positive rates (both 2.5%) for the prediction of an "increased risk" result. When both of the ultrasound markers (DV and TR) were positive, the combined test risk was consistently above 1 in 300 (PPV, 100%; Table 3).

Discussion

Antenatal ultrasound markers have been used for trisomy 21 screening for over two decades [2]. First-trimester fetuses with trisomy 21 typically exhibit increased NT

Table 3. Efficacy of ultrasound parameters for the prediction of increased risk (>1 in 300) in first-trimester screening test*

	Sensitivity	Specificity	PPV	NPV	FNR	FPR	AR	LR (+)	LR (–)
Abnormal DV flow	33.3	97.5	85.7	76.4	66.6	2.5	77.5	13.3	0.68
TR	27.7	97.5	83.3	75	72.2	2.5	75.8	11.1	0.74
Abnormal DV flow+TR	26.3	100	100	73.5	73.6	0	75.8	–	0.73

**Data are presented as percentages. PPV=positive predictive value; NPV=negative predictive value; FNR=false-negative rate; FPR=false-positive rate; AR=accuracy rate; LR=likelihood ratio; +=positive; −=negative; DV=ductus venosus; TR=tricuspid regurgitation.*

and an absence of nasal bone in about 75% and 70% of cases, respectively. Recent research has led to the inclusion of various ultrasound markers for first-trimester trisomy 21 detection [2]. DV Doppler waveform analysis and TR can have significant implications within this context. The first-trimester combined test (NT plus PAPP-A plus free β-hCG) is widely employed as a screening tool, with considerable accuracy [8,13]. DV and TR studies are usually reserved for determining the need for invasive testing in intermediate-risk pregnancies (i.e. 1 in 100 to 1 in 1,000).

We speculated that DV and/or TR ultrasound studies at 11–14 weeks of gestation could predict a high-risk combined test result (i.e. >1 in 300), and the present pilot study was, therefore, performed. Pregnancies with abnormal karyotypes or fetal malformations were excluded, as the aim was to obtain preliminary data based on a limited number of women that could provide the basis for further research. The results indicated high specificities and PPVs with low false-positive rates for DV and TR investigations. Neither marker appeared to be superior to the other. Moreover, all pregnancies with an abnormal DV flow accompanied by TR fell in the risk-group (>1 in 300), yielding a PPV of 100% and a false-positive rate of 0%. The combined screening method, thus, provided no additional information when both DV and tricuspid flow were abnormal.

The low sensitivity observed in the current study was a matter for concern. Sensitivity is generally expected to be low when specificity is high, as in our data. Low sensitivity can also occur if the cut-off value is set too high. However, we believe that reducing the cut-off value in this study would be clinically unrealistic, and would increase the false-positive rate. More false-positive results would suggest more high-risk tests (that are in fact below the cut-off value), thus leading to more invasive tests.

A possible limitation in this study was its inability to directly investigate the relationship between DV flow or TR and fetal karyotype abnormalities. The dataset had insufficient power to predict abnormal karyotype results. The relatively high rates of abnormal DV flow and TR (12% and 10%, respectively) in a healthy population could also be a matter of concern. Abnormal DV flow has been reported in 3–5% of otherwise normal fetuses [4,11,14], while the corresponding rate for TR is 3–9% [4,10,12]. The relatively high rates encountered in the current study can be attributed to the inclusion of a selected invasively karyotyped population. This could have caused a bias in the results, as PPV increases with the prevalence of a given condition. However, the low false-positive rate should be unaffected, as specificity does not depend on the prevalence. The reason why some otherwise healthy fetuses show abnormal DV Doppler waveforms at 11–14 weeks' gestation is unknown; possible causes include cardiac preload or afterload changes over time, or relatively distal communication of the DV with the inferior vena cava in those fetuses.

Matias et al [11] were the first to emphasize the relationship between abnormal *a* wave flow in the DV and chromosomal abnormalities. About 90% of chromosomally abnormal fetuses showed an abnormal DV flow pattern, indicating a role for DV flow Doppler studies in reducing false-positive results and decreasing unnecessary interventions. The feasibility of DV waveforms has been investigated in several other studies [14–21]. An abnormal DV flow has been found to be particularly valuable in pregnancies with an abnormal NT measurement. The combined use of DV flow and NT has demonstrated 95% sensitivity, with a 0.08 negative likelihood ratio [14].

The tricuspid valve can be adequately observed during 11–14 week ultrasound scans in 96–98% of the general population, depending on the instrument used and the technical skill of the sonographer [10,12]. TR during the first-trimester scan seems to be significantly associated with karyotype abnormalities. TR is also a predictor of fetal cardiac pathology, even in the absence of chromosomal defects [10,12]. However, the definition of TR has not been standardized; some authors use a minimum regurgitation velocity of 80 cm/s [13,22], whereas others have used values >60 cm/s as "abnormal". In the current study, abnormal tricuspid flow was

regarded as a velocity of > 60 cm/s for maximal regurgitation.

Data on the combined use of DV flow and TR in first-trimester fetuses are scarce. A multicenter study [8] classified pregnancies into three groups, depending on the first-trimester screening test results: high risk (> 1 in 100), moderate risk (1 in 100 to 1 in 1,000), and low risk (< 1 in 1,000) pregnancies. Women in the "high-risk" group were directly offered invasive testing, whereas "low-risk" pregnancies were observed expectantly. First-trimester ultrasound markers (nasal bone, DV flow, and TR studies) were reserved for "moderate" risk women, and the risk was modified accordingly. The decision to perform further invasive testing, therefore, depended on these ultrasound markers. This two-step (contingent) evaluation was associated with a sensitivity of over 90% for the detection of trisomy 21. Interestingly, the independent sensitivities of abnormal DV flow and TR were also high (94.2% and 91.7%, respectively). In the current study, all women with an abnormal DV flow ($n = 7$) and TR ($n = 6$) were offered invasive testing, although the final decision did not depend on these ultrasound parameters, and the physician in charge of the woman was blinded to the DV flow and TR results in most cases. Abnormal DV flow and TR were associated with increased risk in six out of seven (86%) and five out of six (83%) women, respectively. Interestingly, none of the women who were not initially offered invasive testing (and, therefore, excluded from the study) had abnormal DV or tricuspid flow results (data not shown). The preliminary findings of this study, thus, support the use of these two parameters as a first line of investigation.

Despite these advantages, determination of DV flow and TR in first-trimester fetuses can be technically difficult and time consuming. Although DV Doppler waveforms can be obtained relatively easily, tricuspid valve studies are difficult and involve a learning curve. Determination of these two markers has been suggested to require expert skills and training, thus restricting their routine use in an unselected population [5]. However, intra- and interobserver values of DV flow have been reported to be satisfactory in at least one study [23]. Similarly, tricuspid flow could be detected in 98.8% of cases in a study that included 12 obstetricians experienced in fetal cardiac scanning [10]. The same study also examined the consensus among pediatric cardiologists and obstetricians performing the scan; there was an 88% agreement rate when TR was positive, and a 97% agreement rate in the absence of TR [10].

In conclusion, abnormal DV flow and TR detected during the 11–14-week scan is associated with low sensitivity but high PPV and low false positivity for predicting increased risk identified by the routine combined screening test for trisomy 21. The utility of DV and tricuspid flow as initial markers of trisomy 21 risk in unselected pregnancies warrants further investigation.

References

1. McGee DC. Evaluation of first-trimester tricuspid regurgitation for Down syndrome screening. *J Perinat Neonatal Nurs* 2008;22:282–90.
2. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 2004;191:45–67.
3. Flood K, Malone FD. Screening for fetal abnormalities with ultrasound. *Curr Opin Obstet Gynecol* 2008;20:139–45.
4. Malone FD, D'Alton ME; Society for Maternal-Fetal Medicine. First-trimester sonographic screening for Down syndrome. *Obstet Gynecol* 2003;102:1066–79.
5. Nicolaides KH. First-trimester screening for chromosomal abnormalities. *Semin Perinatol* 2005;29:190–4.
6. Ukudeeva A, Ilhan AH, Kavak ZN, Pekin T, Gokaslan H. Comparing the first trimester and second trimester screening programmes for the screening of Down's syndrome. *Turkiye Klinikleri J Gynecol Obst* 2003;13:194–8.
7. Zournatzi V, Daniilidis A, Karidas C, Tantanasis T, Loufopoulos A, Tzafettas J. A prospective two years study of first trimester screening for Down syndrome. *Hippokratia* 2008;12:28–32.
8. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005;25:221–6.
9. Falcon O, Auer M, Gerovassili A, Spencer K, Nicolaides KH. Screening for trisomy 21 by fetal tricuspid regurgitation, nuchal translucency and maternal serum free beta-hCG and PAPP-A at 11^{+0} to 13^{+6} weeks. *Ultrasound Obstet Gynecol* 2006; 27:151–5.
10. Falcon O, Faiola S, Huggon I, Allan L, Nicolaides KH. Fetal tricuspid regurgitation at the 11^{+0} to 13^{+6}-week scan: association with chromosomal defects and reproducibility of the method. *Ultrasound Obstet Gynecol* 2006;27:609–12.
11. Matias A, Gomes C, Flack N, Montenegro N, Nicolaides KH. Screening for chromosomal abnormalities at 10–14 weeks: the role of ductus venosus blood flow. *Ultrasound Obstet Gynecol* 1998;12:380–4.
12. Faiola S, Tsoi E, Huggon IC, Allan LD, Nicolaides KH. Likelihood ratio for trisomy 21 in fetuses with tricuspid regurgitation at the 11 to 13^{+6}-week scan. *Ultrasound Obstet Gynecol* 2005;26:22–7.
13. ACOG Committee on Practice Bulletins. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. *Obstet Gynecol* 2007;109:217–27.
14. Mavrides E, Sairam S, Hollis B, Thilaganathan B. Screening for aneuploidy in the first trimester by assessment of blood flow in the ductus venosus. *BJOG* 2002;109:1015–9.
15. Oh C, Harman C, Baschat AA. Abnormal first-trimester ductus venosus blood flow: a risk factor for adverse outcome

in fetuses with normal nuchal translucency. *Ultrasound Obstet Gynecol* 2007;30:192–6.
16. Maiz N, Valencia C, Emmanuel EE, Staboulidou I, Nicolaides KH. Screening for adverse pregnancy outcome by ductus venosus Doppler at $11–13^{+6}$ weeks of gestation. *Obstet Gynecol* 2008;112:598–605.
17. Borrell A, Gonce A, Martinez JM, Borobio V, Fortuny A, Coll O, Cuckle H. First-trimester screening for Down syndrome with ductus venosus Doppler studies in addition to nuchal translucency and serum markers. *Prenat Diagn* 2005;25:901–5.
18. Antolín E, Comas C, Torrents M, et al. The role of ductus venosus blood flow assessment in screening for chromosomal abnormalities at 10–16 weeks of gestation. *Ultrasound Obstet Gynecol* 2001;17:295–300.
19. Murta CG, Moron AF, Avila MA, Weiner CP. Application of ductus venosus Doppler velocimetry for the detection of fetal aneuploidy in the first trimester of pregnancy. *Fetal Diagn Ther* 2002;17:308–14.
20. Borrell A, Martinez JM, Serés A, Borobio V, Cararach V, Fortuny A. Ductus venosus assessment at the time of nuchal translucency measurement in the detection of fetal aneuploidy. *Prenat Diagn* 2003;23:921–6.
21. Zoppi MA, Putzolu M, Ibba RM, Floris M, Monni G. First-trimester ductus venosus velocimetry in relation to nuchal translucency thickness and fetal karyotype. *Fetal Diagn Ther* 2002;17:52–7.
22. Nicolaides KH, Azar G, Bryne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *BMJ* 1992;304:867–9.
23. Prefumo F, De Biasio P, Venturini PL. Reproducibility of ductus venosus Doppler flow measurements at 11–14 weeks of gestation. *Ultrasound Obstet Gynecol* 2001;17:301–5.